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Aggressiveness of *Phytophthora medicaginis* on chickpea: Phenotyping method determines isolate ranking and host genotype-by-isolate interactions

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Abstract

Phytophthora medicaginis causing Phytophthora root rot of chickpea (*Cicer arietinum*) is an important disease, with genetic resistance using *C. arietinum* × *Cicer echinospermum* crosses as the main disease management strategy. We evaluated pathogenic variation in *P. medicaginis* populations with the aim of improving phenotyping methods for disease resistance. We addressed the question of individual isolate aggressiveness across four different seedling-based phenotyping methods conducted in glasshouses and one field-based phenotyping method. Our results revealed that a seedling media surface inoculation method used on a susceptible *C. arietinum* variety and a moderately resistant *C. arietinum* × *C. echinospermum* backcross detected the greatest variability in aggressiveness among 37 *P. medicaginis* isolates. Evaluations of different components of resistance, using our different phenotyping methods, revealed that differential pathogen–isolate reactions occur with some phenotyping methods. We found support for our hypotheses that the level of aggressiveness of *P. medicaginis* isolates depends on the phenotyping method, and that phenotyping methods interact with both isolate and host genotype reactions. Our cup-based root inoculation method showed promise as a non-field-based phenotyping method, as it provided significant correlations with genotype–isolate rankings in the field experiment for a number of disease parameters.

KEYWORDS
pathogenic variation, quantitative resistance, resistance breeding, root disease

1 | INTRODUCTION

Chickpea is an important grain legume, with mean global annual production third highest after peas and beans (Merga & Haji, 2019). Chickpea is a relatively recent crop in Australia, with the first commercial production occurring in the late 1970s. During the period 1996–2005 Australia accounted for 2% of world production (Knights et al., 2007), but by 2013–2017 it was the second largest producer after India and produced half of the world's exported chickpeas (Merga & Haji, 2019).

Phytophthora root rot (PRR), caused by the oomycete *Phytophthora medicaginis*, is a major root disease of chickpea in Australia (Li et al., 2015; Schwinghamer et al., 2011). Chickpea in eastern Australia is typically grown under dryland conditions in vertisol soil types with high clay contents. Chickpea is one of the few winter pulse crop options in north-eastern Australia; however, subsoil constraints in this area often contribute to poor drainage following saturating rainfall events that favour the pathogen and disease development (Dang et al., 2010; Knights et al., 2007; Schwinghamer et al., 2011). Seed treatment with metalaxyl provides up to 6–8 weeks control but protection does not last the full season (authors' unpublished results). The testing of soil samples for the presence of *P. medicaginis* DNA, to determine inoculum-based disease risk, showed that low risk sites could not be reliably detected (Bithell et al., 2021). The absence of effective site selection and in-crop management options have led to PRR receiving the highest soilborne disease priority of the Australian National Chickpea Breeding Program since the 1990s (Singh et al., 1994). The most effective source of PRR resistance for chickpea is from a wild relative of chickpea, *Cicer echinospermum*, which confers a complex form of quantitative resistance to *P. medicaginis* (Amalraj et al., 2019; Knights et al., 2008).

Phytophthora pathogen populations are known to be variable, and in similar homothallic oomycetes such as *P. sojae*, differences among pathogen isolates are due to the presence of avirulence genes interacting with specific resistance genes in the soybean host, *Glycine max*. This results in large numbers of different pathogen races due to coevolution in a gene-for-gene manner (Drenth et al., 1996; Meng et al., 1999). Different soybean genotypes may also possess different levels of horizontal resistance to *P. sojae* in addition to variation in isolate aggressiveness (Karhoff et al., 2019). These levels of genetic variability, in addition to environmental and experimental variability, require robust tools for accurate assessment of resistance among germplasm in breeding programmes.

Studies of *P. medicaginis* have identified a range of isolate aggressiveness. In a study of *P. medicaginis* isolates sourced from lucerne (18) and chickpea (12) in Australia, disease reactions on nine clonal lucerne genotypes showed an isolate aggressiveness continuum but also expressed some specific interactions between some isolates and lucerne clones (Liew & Irwin, 1997). More recently, Du et al. (2013) evaluated four *P. medicaginis* isolates on three chickpea genotypes and identified a more aggressive isolate, and among two isolate treatments on 16 *C. arietinum* genotypes identified some isolate-specific genotype reactions. These studies indicate that aggressiveness among *P. medicaginis* isolates across host genotypes is complex and can provide genotype-by-isolate (G*I) interactions.

Host–pathogen interactions are also strongly influenced by the environment in addition to host genotype. Phenotyping results obtained from the glasshouse (oospores mixed with potting mix at sowing) and a field experiment with natural inoculum on the same set of genotypes, identified different resistance responses in chickpea to *P. medicaginis* (Dale & Irwin, 1991a). Similarly, field phenotyping over two seasons of chickpea recombinant inbred line populations for *P. medicaginis* resistance found significant genotype-by-environment (G*E) interactions (Amalraj et al., 2019). Because introgression of *P. medicaginis* resistance is fundamental to obtain resistance in Australian commercial cultivars of chickpea, reliable methods of phenotyping chickpea × *C. echinospermum* crosses are paramount.

The overall objective of this study was to assess the variability of rankings of *P. medicaginis* isolate aggressiveness across multiple phenotyping systems using chickpea varieties and chickpea × *C. echinospermum* crosses. We specifically sought to test if (a) aggressiveness reactions on a susceptible chickpea variety and a chickpea × *C. echinospermum* cross would differ among a population of 37 different *P. medicaginis* isolates; (b) the composition of isolate mixtures would affect assessment of resistance; (c) G*I interactions are present in glasshouse and/or field testing; (d) different phenotyping methods affect rankings of progeny resistance levels or isolate aggressiveness; and (e) different phenotyping methods affect the G*I interactions.

An in-depth understanding of pathogen variability, phenotyping methods and interactions of pathogen isolates with host genotypes is paramount to achieve genetic gain for resistance in chickpea against *P. medicaginis*.

2 | MATERIALS AND METHODS

2.1 | Isolate culturing

Thirty-seven isolates of *P. medicaginis* were obtained from a number of sources and their pathogenicity to chickpea had been confirmed previously following Koch's postulates (Table 1). Isolates were stored on low-strength V8 medium (100 ml V8 juice, 10 g agar, 2.5 g calcium carbonate, 900 ml Milli-Q water) under water in bottles at 4°C. Prior to annual inoculum production, each isolate was passaged through plants in a glasshouse using the susceptible chickpea cultivar Sonali. Surface-sterilized Sonali seeds were individually pushed into a premoistened 44-mm diameter peat plug (Jiffy 7, product code 32170142, Jiffy Products International AS) to a depth of 12 mm from the top of the seed and placed in a 180-ml plastic cup (75 mm high; Brighton Co.). Using low-strength V8 medium, an oospore suspension was prepared by macerating cultures with a hand-held Braun 600 W blender, then added to flooded (Milli-Q water) cups of seedlings, and cups were drained after 48 h. After the observation of wilting, chlorosis and canker development on the seedlings, stem tissue at the margin of the canker was used to isolate the pathogen on corn meal agar. Cultures were hyphal tipped and then grown on low-strength V8 medium. Subcultures of these freshly passaged isolates were used to produce 90 mm-diameter Petri dish cultures of each isolate in the dark at 21–23°C.

Agar from plate cultures of *P. medicaginis* cultures that were a minimum of 6 weeks old was mixed with Milli-Q water (10% vol/vol) and macerated using a hand-held Braun 600 W blender for approximately 3 min. Average oospore concentrations were determined

TABLE 1 Information on 38 *Phytophthora medicaginis* isolates used in study including 37 isolates in the aggressiveness range glasshouse experiment with treatment (Trt.) code, isolate identification (ID) number, collection date, source from either isolation from plant material or baited from soil sample, host species for isolates from plant material (*Medicago sativa*, Lu; *Cicer arietinum*, Cp) or plant species present where soil samples were collected, location of plant or soil collection (New South Wales, NSW; Queensland, Qld) in Australia and number of oospores per plate used in the aggressiveness range experiment

Trt. code	Isolate ID	Collection date	Source	Species	Location	No. oospores/ plate
1	4019	01/04/2004	Plant	Lu	UQ5750, Gatton Qld	36,800
2	4021	01/04/2004	Plant	Lu	UQ5751, Gatton Qld	353,689
3	4026	23/05/2001	Plant	Lu	UQ5618, Gatton Qld	78,000
4	4027	23/05/2001	Plant	Lu	UQ5617, Gatton Qld	34,800
5	4046	03/08/2005	Plant	Cp	Moree, NSW	353,600
6	4065	13/09/2005	Plant	Cp	Tamworth, NSW	63,200
7	4091	12/10/2005	Plant	Cp	Tamworth, NSW	90,000
8	5601	05/07/2012	Plant	Cp	Pallamallawa, NSW	263,200
9	7492	08/09/2015	Plant	Cp	MacAlister, Qld	248,400
10	7612	03/08/2013	Soil	Cp	Amatree, NSW	246,000
11	7614	07/08/2013	Soil	Cp	Yallaroi, NSW	241,600
12	7616	07/08/2013	Soil	Cp	Yallaroi, NSW	132,000
13	7620	07/08/2013	Soil	Cp	North Star, NSW	234,800
14	7622	08/08/2013	Soil	Cp	Garah, NSW	106,000
15	7624	18/09/2013	Soil	Cp	Warra, NSW	130,400
16	7625	03/10/2013	Soil	Cp	Moree, NSW	404,800
17	7627	03/10/2013	Soil	Cp	Moree, NSW	491,178
18	7628	05/03/2014	Soil	Cp	North Star, NSW	184,000
19	7793	20/08/2015	Plant	Cp	Coonamble, NSW	937,378
21	7801	11/09/2015	Plant	Cp	Narrabri, NSW	103,756
22	7802	18/09/2015	Plant	Cp	Narrabri, NSW	347,556
23	7803	18/09/2015	Plant	Cp	Maules Creek, NSW	833,622
–[a]	7830	18/08/2014	Soil	Lu	Biloela, Qld	–
24	7831	09/09/2014	Soil	Cp	Mungallala, Qld	658,311
25	7833	09/09/2014	Soil	Cp	Mungallala, Qld	134,422
26	7834	09/09/2014	Soil	Cp + *Vicia villosa* subsp. *dasycarpa*	Mungallala, Qld	548,422
27	7838	10/09/2014	Soil	Cp	Dulacca, Qld	154,356
28	7840	21/08/2015	Soil	Cp	Goondiwindi, Qld	112,444
29	7842	22/10/2014	Soil	Luc	Wagga Wagga, NSW	61,333
30	7846	25/06/2014	Soil	Cp	Sandy Creek, NSW	147,200
31	7902	10/03/2008	Plant	Cp	Hermitage, Qld	33,733
32	7904	10/03/2008	Plant	Cp	Hermitage, Qld	82,800
33	7919	05/04/1995	Plant	Lu	Gatton, Qld	64,400
34	7923	03/10/2013	Plant	Cp	Moree, NSW	918,978
35	7925	02/08/2014	Plant	Cp	Bellata, NSW	182,978
36	9470	10/08/2016	Plant	Cp	Tamworth, NSW	203,422
37	1129-1	24/10/1988	Plant	Cp	Yetman, NSW	23,511
38	943c-1	08/09/1987	Plant	Cp	Trangie, NSW	16,356

Note: The 10 *P. medicaginis* breeding isolates used in the mixture treatments are underlined.

[a]Isolate not included in aggressiveness range glasshouse experiment.

using counts under a 20 × 50 mm coverslip to prepare inoculum containing equal oospore concentrations.

2.2 | Aggressiveness range glasshouse experiment

To test for differences in isolate aggressiveness, a "peat surface" inoculation adjacent to the stem base assay was used on two genotypes: the moderately resistant breeding line 04067-81-2-1-1(B) (a backcross derivative from *C. echinospermum*) and the susceptible chickpea cultivar Rupali. The experiment comprised 37 *P. medicaginis* isolates, one mixture of 10 *P. medicaginis* isolates and an uninoculated control treatment (Table 1). The experimental design was a randomized complete block comprising 312 (39 isolate treatments × 2 genotypes × 4 replicates) experimental units. The 10 isolates in the mixture were the same as used in the Australian national chickpea breeding programme from 2013 to 2017 (Table 1) and are hereafter described as the 10 Pm breeding isolates.

Surface-sterilized seeds were set up in peat plugs inside cups as previously described, and each cup had a single 7-mm aperture drain hole on the side wall 12 mm above the base. Ten days after set-up, seedlings of each genotype with three or four nodes were selected and flooded to the surface of the medium with filtered Milli-Q water. They were then inoculated adjacent to the stem base with a 2 ml suspension containing 1000 oospores/seedling, or the control suspension (blended V8 agar in suspension).

The plants were propagated in a temperature-controlled glasshouse on a 22°C night/27°C day cycle. Then after the addition of a nonholed second cup underneath, cups had water added to bring the flooding level to approximately 5 mm above the surface of the peat medium; this flooding level was maintained for 48 h, after which the cups were drained by removal of the second cup. Cups were then watered to a target tared weight of 43 g of water per peat plug to provide saturation (0 kPa) and maintained above field capacity by hand watering at 2- to 3-day intervals over the experimental period. As a parameter of root function, plant water use was calculated as the weight consumption change value by subtracting the target watered weight from the subsequent recorded weight for each cup in each watering interval. Water consumption per 24-h period was then calculated for each interval by dividing the consumption change value by the number of days in each interval. Cups were fertilized once a fortnight with 1 ml of nutrient solution containing 1 g/L of NPK 25:5:8.8, S 4.6, Mg 0.5, Fe 0.18, B 0.005, Cu 0.005, Zn 0.004 and Mo 0.001.

To measure the relative differences in isolate aggressiveness, plants were assessed for the presence of chlorosis, stem cankers and plant death symptoms three times a week. When plants died, the peat medium was washed from the roots; if a stem canker was observed on the epicotyl, the length (mm) of the canker was measured from the start of the epicotyl stem region at the juncture with the cotyledons to the margin of the canker on the stem as a visual assessment of aggressiveness. Any cotyledons were then removed with a scalpel and the plants dried at 65°C for 72 h and weighed. The experiment ended 34 days after inoculation. Plants that had not died were prepared, dried, measured and weighed.

2.3 | Genotype-by-isolate interaction glasshouse experiment

A peat root inoculation assay was used to test for the presence of G*I with eight diverse *P. medicaginis* isolates (5601, 7616, 7830, 7838, 7842, 7902, 7919 and 9470) and a control treatment. The isolates were tested across 11 genotypes listed in Figure 1, including two chickpea × *C. echinospermum* crosses (04067-81-2-1-1(B) and CICA1328) and a *C. echinospermum* accession (ILWC246). The experimental design was a randomized complete block comprising 297 (9 isolate treatments × 11 genotypes × 3 replicates) experimental units.

Surface-sterilized seeds of each genotype were placed individually in a premoistened peat plug placed in a 180-ml cup. Ten days after setup when the roots of all genotypes had grown through the base of the peat plug and tops were at the 3–4-node growth stage, the seedlings in peat plugs were placed in an intact 180-ml cup and saturated to run-off by the repeated addition of 5–10 ml of Milli-Q water over the surface of the peat plug at 5–10 min intervals. When free water was visible in the bottom of each cup and the peat plug did not absorb any more water, the seedling–peat plug unit was removed from the cup and placed into a new cup (with 7-mm side wall hole as previously described) with a 5 ml suspension containing 2000 oospores. Blended V8 agar in suspension was used as the control treatment. The cups were then placed into a randomized complete block layout in an air-conditioned glasshouse, with temperatures ranging from 6°C overnight to 30°C during the day.

Isolate aggressiveness was assessed by observing chlorosis, stem cankers and plant death symptoms, and measuring canker length and dry weight as per the earlier experiment, with the experiment ending 46 days after inoculation. Plants were watered to saturation (0 kPa, 54 g water per peat plug) and maintained above field capacity by watering two or three times a week depending on water use and fertilized as described previously. The control and isolate 5601 treatments across all nine genotypes ($n = 36$ cups per isolate) were weighed before and after each watering event.

2.4 | Phenotyping method experiment

To test the effect of different phenotyping methods on isolate rankings and G*I, the following experiment was performed. The experiment comprised three isolate treatments of two isolates (5601 and 7842) or a control, six genotypes (Rupali (A), 04067-81-2-1-1(B), Yorker, Genesis 114, CICA1328 and ILWC246) and four phenotyping methods: (a) peat surface inoculation adjacent to the stem base method; (b) peat root inoculation method; (c) peat and sand surface inoculation adjacent to the stem base method; and (d) sand surface inoculation adjacent to the stem base method. Sand was selected as

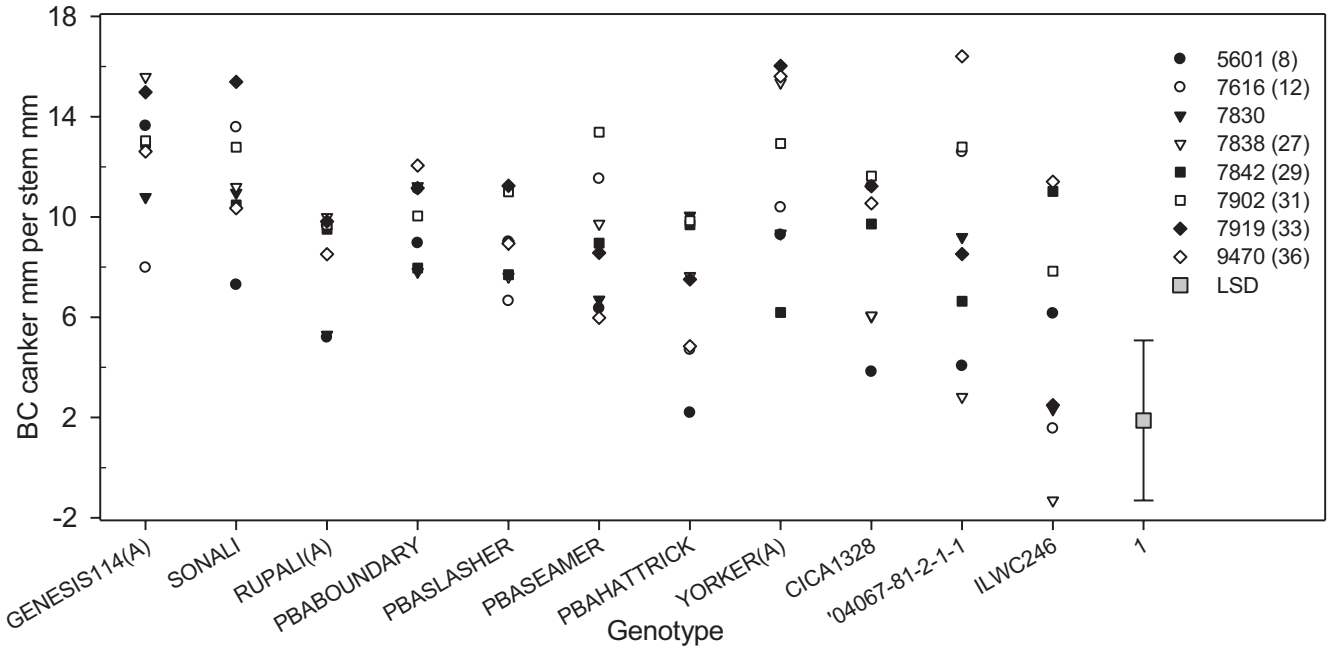

FIGURE 1 Isolate-by-genotype interaction glasshouse experiment. *Phytophthora medicaginis* isolate by peat medium root-inoculated chickpea genotype interaction for Box-Cox (BC)-transformed canker length mm per stem mm. LSD value of 6.39 presented as an error bar; the percentage of total variance value for this interaction is 26.7. Genotypes are ordered on the basis of established field resistance rankings, with the most susceptible genotypes to the left and most resistant to the right. Isolate treatment codes for the Aggressiveness range glasshouse experiment are provided in parentheses for comparison with Figure 2

a medium to provide a contrasting matric potential to that of peat. The experimental design was a randomized complete block comprising 288 (3 isolate treatments × 6 genotypes × 4 methods × 4 replicates) experimental units.

The medium was prepared for peat surface and peat root methods as described for the two earlier experiments. For the peat and sand surface method, the peat plug was saturated as described previously and then 57 g sand was poured over the peat plug. For the sand surface method, cups were prepared with 157 g of the same sand as above. Surface-sterilized seed of the six genotypes were sown in the media to a depth of 12 mm and the cups were placed in a controlled environment with a 12:12-h light–dark cycle with 11°C night and 19°C day. When seedlings were 10 days old, a second cup with no drainage holes was put under the first cup and the medium saturated with Milli-Q water. Then two 1 ml solutions containing 500 oospores each were applied to each cup adjacent to the stem base for the 5601 and 7842 treatments for the three media surface inoculation methods. The control treatment cups received two 1 ml Milli-Q water only inoculations. Further water was then added to each cup as described for the aggressiveness range experiment. For the peat root inoculation, the seedling roots were placed into a cup contained a 1000 oospore/2 ml suspension or water only for the control treatment.

Isolate aggressiveness was assessed by observing chlorosis, stem cankers and plant death symptoms, and measuring canker length and dry weight as per the earlier experiments, with the experiment ending 35 days after inoculation. Plants were watered to saturation (0 kPa) and for each treatment the matric potential was maintained above −14 kPa by watering individual cups to weight two or three times a week depending on water use. The plants were fertilized as previously described.

2.5 | Isolate aggressiveness field experiment

The presence of G*I under field conditions was tested using six isolate treatments, consisting of four separate individual isolates (4021, 5601, 7842 and 9470), and two isolate mixture treatments, both of which contained the 10 Pm breeding isolates. The first mixture "Equal Oospore" treatment comprised equal oospore concentrations of each of the 10 isolates, and the second mixture "Equal Plate" treatment comprised a suspension prepared from equal numbers of Petri dishes of the 10 isolates. The oospore concentrations of the isolates in the "Equal Plate" treatment are provided in Table S1. Isolates were tested on two genotypes, 04067-81-2-1-1(B) and Yorker. The experiment was a randomized complete block design comprising 56 (7 isolate treatments × 2 genotypes × 4 replicates) experimental units, and was established at the Hermitage Research Facility, Warwick, Queensland, Australia.

The experiment was planted on 25 June 2018 with 04067-81-2-1-1(B) (20.8 g/100 seed) and Yorker (22.9 g/100 seed) treated with P-Pickle-T (360 g/L thiram and 200 g/L thiabendazole) applied as a suspension at 10 ml/kg of seed. P-Pickle-T seed treatment provides protection against non-oomycete pathogens and is an advised standard industry practice. Seed weight and germination data were used to calculate the required sowing rate to establish a

field density of 35 plants/m^2. The soil type was a grey vertosol. Each plot was surrounded with four buffer rows sown with metalaxyl-treated seed (350 g/L Metalaxyl-M, 75 ml/100 kg seed) to prevent the potential spread of *P. medicaginis* inoculum between plots. Four-row 14.8 m^2 plots were sown with a cone seeder, equipped with separate drums for applying *P. medicaginis* inoculum at a concentration of 565 oospores/ml at 77 ml/m row length and *Mesorhizobium ciceri* (CC1192) rhizobia. The growing season was drier than usual, with a total of 53 mm of rain from June to September (long-term average for this period is 121 mm), but October had high rainfall with 128 mm largely over the last three weeks of the month. Supplementary irrigation provided from 11 to 13 September using dripper tape (T-tape, Rivulas Irrigation) delivered 27 mm in total.

Data, including plant counts, disease assessments and yield were obtained from the middle two rows of each four-row plot. Seedling emergence counts were made on 31 July, and counts of the total number of plants, number of dead and number of chlorotic plants were made on 28 August, 17 September, 8 October and 19 November. On 12 September a count of the total number of dead and chlorotic plants was made. To provide a multi-assessment-based disease parameter, the area under the disease progress stairs (AUDPS) (Simko & Piepho, 2011) was calculated as separate parameters for the proportion of dead plants per plot and plants with symptoms (chlorotic plus dead) per plot for disease assessments. Using 3-hourly temperature records from a soil depth of 100 mm recorded 750 m from the field experiment (Bureau of Meteorology Station # 41525), thermal time accumulation (°Cd) was calculated using a T_b of 5°C and T_{max} of 35°C, and daily accumulation was calculated for the daily maximum and minimum temperature values between 5 and 35°C. Average daily thermal time between disease assessments increased from July to August 9.3°Cd, August to early September 11.9°Cd, mid-September 15.2°Cd and 15.8°Cd from September to October.

To determine the effect of *P. medicaginis* on root development and pathogen multiplication, on 13 November a 300 mm row section was sampled from the southern end of each of the two middle rows of each plot by taking four separate 45 mm diameter 100 mm depth soil cores, collected from each side of each row section approximately 20 mm from the closest stem base, and placed in separate bags and dried at 40°C for 72 h. Plants and roots were then dug using a garden fork to a depth of 200 mm, the number of plants in each row section sample was counted, the tops cut from the roots above the lowest branching point on the stem, and the stem base–root samples bagged. In the laboratory the root base and attached roots were removed from the stem base by cutting through the stem base at the lower juncture of the first branching point on the stem, and the stem base–root samples were hand washed to remove any adhering soil. The number of tap roots showing lesions where the apex of the tap root had rotted away was counted and samples dried at 65°C for 72 h to determine stem base–root dry weights. After the drying of the soil core samples at 40°C, a 400 g subsample was sent to the Root Disease Testing Service at the South Australian Research and Development Institute (Adelaide, Australia) to measure the *P. medicaginis* soil DNA concentration as described by Bithell et al. (2021).

2.6 | Analysis

Residuals were examined by visual evaluation of quantile:quantile plots; if necessary, data were appropriately transformed to meet requirements for residuals to be normally distributed. Data were analysed with analysis of variance (ANOVA). No disease symptoms were observed in control treatments in any of the experiments, and so to focus on differences among isolates the control treatments were not included in the analysis.

Survival analysis was carried out on the number of days from inoculation to the presence of chlorosis, canker or death symptoms to provide predicted means to 50% incidence (Therneau, 2020). Between-experiment result comparisons were carried out with correlations using mean nontransformed data from each experiment, except for day to symptom intervals which used the predicted mean values from the survival analysis and square root-transformed *P. medicaginis* DNA copy numbers and AUDPS values from the field experiment; the significance ($p < 0.05$) of correlation coefficients were assessed using a two-tailed *t* test.

Statistical analyses were carried out with GenStat (VSN International) and R (R Core Team, 2020).

3 | RESULTS

3.1 | Aggressiveness range glasshouse experiment

This experiment tested for differing isolate aggressiveness on two genotypes with contrasting *P. medicaginis* resistance. A two-tailed *t* test indicated there were significant ($p < 0.001$, $df = 36$) differences in the number of oospores produced among isolates (Table 1). For all eight parameters there was a significant range ($p < 0.01$) in isolate aggressiveness (Table 2). Results demonstrated a continuum of aggressiveness reactions ranging between weakly pathogenic isolates and more aggressive isolates. Isolate 4021 was weakly pathogenic across all parameters, whilst isolate 7624 was one of the most aggressive across most parameters. The aggressiveness of some isolates did not differ from 7624, such as 5601 which only had a less aggressive effect on dry weight than isolate 7624. Other isolates occupied intermediate positions between aggressiveness extremes, for example, isolate 7842 was significantly less aggressive than 7642 for parameter responses including water use, stem relative canker length, seedling length and dry weights but had more aggressive responses than isolate 4021 for all these parameters. This was also the case for isolate 9470 for a differing set of parameters. The aggressiveness of the 10 isolate mixture did not differ significantly from isolate 7624 for most parameters, but the mixture parameter responses were less aggressive than 7624 in terms of water use and seedling dry weight.

TABLE 2 Aggressiveness range glasshouse experiment. Main effects for (a) *Phytophthora medicaginis* isolate (37 individual, 1 mixture) and (b) chickpea genotype (Rupali, 04067-81-2-1-1(B)) results from the peat medium surface inoculation method presented by treatment (Trt.) code, isolate identification (ID) for the parameters predicted mean days to appearance of chlorosis (day Ch), canker (day Ca), or death (day D), plant water use (WU) day over a four-day period before the first dead plant occurred, stem canker length (Ca), stem canker length relative to seedling length (PCA), seedling length (Len) and plant dry weight (DW)

Trt. code	ID	day Ch	day Ca	Day D	WU (g/day)	Ca (mm)	GG PCA (bt)	SQ Len (mm) (bt)	BC DW (g) (bt)
(a) Isolate main effects									
1	4019	11.8	16.7	28.4	7.3	24.9	18.3 (18.2)	11.7 (137)	−6.1 (0.09)
2	4021	21.7	23.0	38.9	8.8	19.0	13.2 (12.1)	13.2 (173)	−3.4 (0.18)
3	4026	11.1	14.4	28.7	7.5	23.1	17.6 (17.5)	11.6 (135)	−5.7 (0.09)
4	4027	14.3	16.8	30.3	7.0	24.1	18.0 (18.0)	11.6 (135)	−6.0 (0.09)
5	4046	11.4	13.7	25.2	6.5	24.0	19.1 (19.1)	11.2 (127)	−6.0 (0.09)
6	4065	11.0	14.1	27.1	6.4	20.0	16.4 (15.8)	11.3 (128)	−5.6 (0.09)
7	4091	11.4	14.5	25.2	6.4	25.6	20.6 (20.7)	11.1 (124)	−6.5 (0.08)
8	5601	10.7	13.7	23.1	5.7	26.8	23.3 (24.0)	10.5 (111)	−6.8 (0.07)
9	7492	15.0	17.7	*	8.6	24.3	16.0 (15.7)	12.9 (166)	−4.6 (0.12)
10	7612	11.4	14.8	27.4	7.3	24.9	18.9 (18.9)	11.6 (134)	−6.5 (0.08)
11	7614	11.0	15.6	28.1	6.1	23.3	20.2 (20.3)	10.9 (118)	−6.7 (0.08)
12	7616	10.9	12.9	27.2	6.4	27.0	20.4 (20.4)	11.5 (133)	−6.5 (0.08)
13	7620	10.9	15.4	24.0	6.3	23.8	20.5 (20.6)	10.8 (116)	−7.2 (0.07)
14	7622	10.9	12.8	27.6	7.0	31.9	23.5 (24.5)	11.1 (124)	−6.8 (0.08)
15	7624	10.7	14.4	22.5	5.6	26.5	25.7 (26.6)	10.0 (100)	−8.5 (0.06)
16	7625	11.0	16.8	28.2	7.4	28.5	22.1 (22.1)	11.3 (129)	−6.7 (0.08)
17	7627	13.4	17.1	30.3	7.0	25.5	18.5 (18.5)	11.7 (138)	−5.4 (0.10)
18	7628	12.8	14.7	25.8	5.9	22.4	19.9 (19.9)	10.5 (111)	−7.3 (0.07)
19	7793	11.0	14.1	27.2	7.0	28.3	21.0 (21.4)	11.9 (143)	−5.8 (0.09)
21	7801	13.0	15.5	26.8	7.5	24.5	19.4 (19.3)	11.4 (129)	−6.7 (0.08)
22	7802	10.9	14.1	27.1	6.0	26.8	21.7 (22.0)	10.7 (114)	−7.2 (0.07)
23	7803	13.4	16.6	29.1	6.9	28.8	21.1 (21.1)	11.7 (137)	−6.1 (0.09)
24	7831	10.7	16.2	28.5	7.2	22.1	16.9 (16.7)	11.4 (132)	−6.8 (0.08)
25	7833	11.0	16.0	22.6	7.3	27.5	20.5 (20.5)	11.6 (135)	−6.0 (0.09)
26	7834	10.9	15.9	25.5	6.8	25.0	19.7 (19.7)	11.3 (128)	−6.5 (0.08)
27	7838	10.9	15.0	26.8	6.0	28.3	23.3 (23.9)	10.6 (114)	−7.5 (0.07)
28	7840	13.8	17.4	29.9	6.7	21.4	19.2 (19.0)	10.9 (120)	−6.5 (0.08)
29	7842	11.4	16.3	29.0	7.4	28.0	21.1 (21.3)	11.4 (130)	−6.0 (0.09)
30	7846	13.0	14.4	27.4	7.9	25.9	17.4 (17.2)	12.2 (148)	−5.2 (0.10)
31	7902	10.9	14.3	28.6	7.1	25.4	21.5 (21.7)	10.7 (115)	−6.6 (0.08)
32	7904	11.0	19.0	32.7	6.7	24.9	20.1 (20.1)	11.1 (122)	−6.6 (0.08)
33	7919	13.0	17.7	26.1	6.1	22.4	20.0 (20.1)	10.8 (118)	−7.0 (0.07)
34	7923	10.7	15.0	25.9	7.0	25.8	21.1 (21.1)	11.0 (123)	−6.9 (0.08)
35	7925	10.7	13.4	28.0	6.2	22.4	18.7 (18.7)	11.0 (121)	−6.5 (0.09)
36	9470	19.9	21.1	34.8	6.8	27.9	21.5 (21.8)	11.3 (128)	−5.8 (0.09)
37	1129-1	10.5	14.1	24.5	5.8	26.6	22.1 (22.5)	10.8 (117)	−6.9 (0.07)
38	943c-1	11.0	22.5	29.3	6.9	23.5	19.6 (19.6)	11.0 (120)	−6.7 (0.08)
39	Mix.	11.6	13.7	29.1	6.9	29.4	22.6 (23.3)	11.0 (121)	−6.9 (0.07)
Isolate error terms		0.57[a]	0.99	4.20	0.62[2]/1.22[3]	2.74/5.40	2.11/4.15	0.51/1.01	0.79/1.57
(b) Genotype main effects									
Rupali		11.2 (0.52[a])	13.2 (0.82)	27.8 (2.38)	7.1	30.6	22.4 (22.1)	11.6 (142)	−5.8 (0.12)
04067-81-2-1-1(B)		13.0 (0.61)	17.1 (1.06)	28.0 (2.64)	6.6	19.9	17.7 (17.1)	10.9 (122)	−6.9 (0.10)
Genotype error terms					0.14[b]/0.28[c]	0.63/1.24	0.48/0.95	0.12/0.23	0.18/0.36

Note: Data transformations indicated as Gaussianized (GG), square root (SQ) and Box-Cox (BC), for transformed data back-transformed (bt) values are presented residual *df* = 225. Error terms, from survival analysis [a]average *SEM*, from analysis of variance *SED*[b]/LSD[c].

*No death.

There were significant ($p < 0.001$) genotype main effects for all parameters, which showed Rupali to have significantly greater susceptibility to *P. medicaginis* isolates as indicated by canker length and stem relative canker length, although Rupali seedlings were significantly longer and heavier than 04067-81-2-1-1(B) and consequently consumed more water (Table 2).

Results from this experiment also provided significant G*I ($p < 0.01$) for five parameters, survival analysis values for days to chlorosis, canker and death, stem relative canker length, seedling length and dry weight. The survival analysis results for days to chlorosis and canker showed that interactions resulted from small groups of isolates being less aggressive on the chickpea × *C. echinospermum* backcross, 04067-81-2-1-1(B), than the majority of the isolates on Rupali (Figure 2a,b). However, in the days to death parameter, there were groups of isolates that caused earlier death on 04067-81-2-1-1(B) than Rupali and vice versa (Figure 2c). For the interaction effects on seedling length where the control showed that Rupali was longer than 04067-81-2-1-1(B), significant separation occurred between isolates that caused smaller lengths on Rupali (7840) and those that caused smaller lengths on 04067-81-2-1-1(B) (7624, 7627, 7831, 7925) (Figure 2d). Seedling length and dry weight values were highly correlated ($r = 0.636$ $p < 0.001$ for two-sided t test) with the seedling dry weight interaction providing similar results to that of the seedling length interaction (not presented). The presence of G*I was also evaluated by nonparametric rank order analysis (data not presented). The rank order determined that isolate rankings were significantly correlated for three parameters—days to chlorosis, water use per day and canker length. For the other five parameters isolate rankings were not significantly correlated.

3.2 | Genotype-by-isolate interaction glasshouse experiment

Testing for G*I interactions for aggressiveness across eight isolates inoculated on roots of 11 genotypes covering a range of *P. medicaginis* resistance revealed that all six parameters provided significant ($p < 0.05$) main effects for both isolate and genotype (Table 3). For days to chlorosis and canker, isolate 7902 had the most rapid symptom development for both parameters, while isolate 5601 had the slowest symptom development, and other isolates had intermediate values. For the canker, seedling length and dry weight-based parameters the rankings of isolates were similar among parameters for the most (7902) and least (5601) aggressive isolates.

There were three significant G*I interactions. The canker length relative to stem length parameter showed the greatest separation ($p < 0.05$) among isolates on two of the most resistant genotypes, a *C. echinospermum* line ILWC246 and a chickpea × *C. echinospermum* backcross 04067-81-2-1-1(B), with less separation on susceptible genotypes (Figure 1). Among some of the moderately susceptible chickpea genotypes (PBA Seamer, PBA HatTrick, Yorker) and the two chickpea × *C. echinospermum* crosses (CICA1328, 04067-81-2-1-1(B)) and ILWC246, there were some G*I interactions. For example, on ILWC246, isolates 7842 and 9470 both caused the largest canker length relative to stem length (approximately 11 mm), but on Yorker isolate 9470 (11.4 mm) was significantly more aggressive than isolate 7842 (6.2 mm). The other significant ($p < 0.001$) interactions evident were for days to chlorosis and days to canker among moderately resistant genotypes such as Yorker (Figure S1). For isolate 5601 on Yorker chlorosis occurred after 20 days, but for PBA HatTrick chlorosis occurred at day 43; similar differences between these two varieties were observed for days to canker.

3.3 | Phenotyping method experiment

Significant differences were observed among the four phenotyping methods ($p < 0.05$) for six of the parameters (Table S2, Table 4a). Both the peat surface and peat root inoculation methods gave significantly more rapid chlorosis and canker symptom development compared to the two other methods (Table 4a). The peat root method had the largest effect on water use. The sand surface method also had a larger effect than the peat surface method, with the sand and peat surface method having the smallest effect. For canker and seedling length, the sand surface method had the smallest effect, but for seedling dry weight the sand and peat media method had a smaller effect than the three other methods.

The phenotyping method significantly interacted with isolate (M*I) for four of eight parameters but there was no significant G*I (Table S2). For days to chlorosis, plants inoculated with isolate 5601 using the peat root method had the fewest days to chlorosis, but for plants inoculated with isolate 7842, the peat root method produced similar values to the sand surface method (Table 4b). For canker length, transformed dry weight and water use, in terms of general effects, the more aggressive isolate 7842 had the least statistical separation among phenotyping methods, while the less aggressive isolate 5601 had more separation among phenotyping methods. For specific effects, isolate 7842 produced longer canker lesions than isolate 5601 for the peat and sand, and sand surface methods. In contrast, for the peat root method only, the seedling dry weight of 5601-inoculated plants were lighter than the 7842-inoculated plants, but the sand surface isolate 7842 inoculation produced lighter plants than isolate 5601. Furthermore, peat root isolate 5601-inoculated plants used less water than all other method and isolate combinations.

There were phenotyping method-by-genotype interactions (M*G) for six parameters (Table S2). For the parameter canker length, there was no significant variation among the four methods for the genotypes Rupali, Yorker and ILWC246, but for the other three genotypes the sand surface method produced smaller cankers than the other methods (Figure 3a). For the water use parameter, values did not differ between the sand surface and peat root methods for Yorker, 04067-81-2-1-1(B), and ILWC246, but water use values did differ between these two methods for the other genotypes (data not presented). The differing interactions between water use and canker size also differed to those for seedling length and dry weight

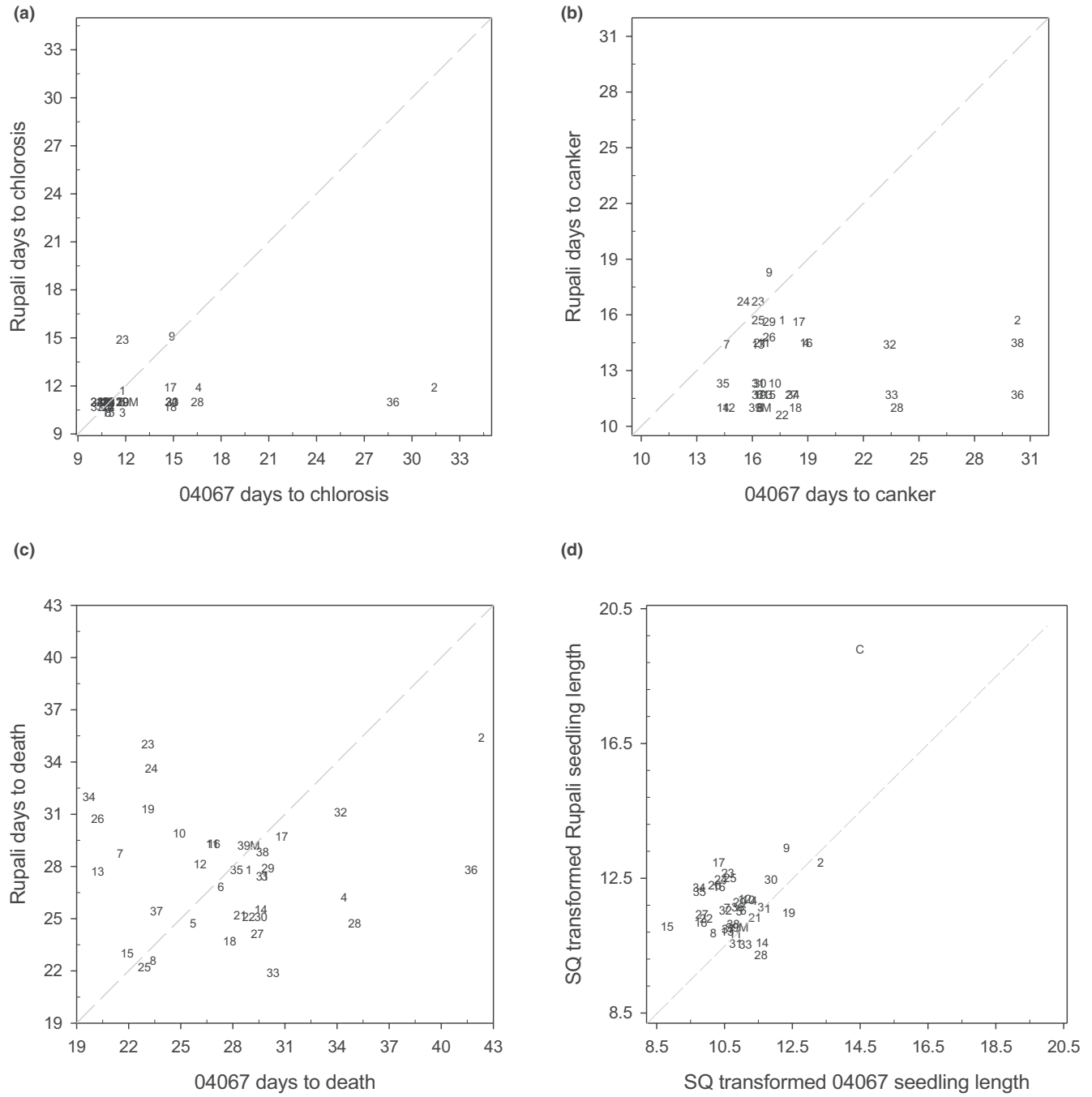

FIGURE 2 Aggressiveness range glasshouse experiment. Plots of parameters with significant chickpea genotype by *Phytophthora medicaginis* isolate interactions for peat medium surface-inoculated Rupali and 04067-81-2-1-1(B) seedlings inoculated with 38 *P. medicaginis* isolate treatments (37 individual isolates, 1 mixture); for analysis of variance output the percentage of total variance (PTV) for each interaction is provided after the LSD value. (a) Interaction of days to chlorosis; (b) interaction of days to canker; (c) interaction of days to death (LSD 7.25); and (d) interaction of square root (SQ)-transformed seedling length (LSD 1.43, PTV 16.3). Numbers in each plot are the isolate treatment codes listed in Table 1. The letter M represents the 10 isolate mixture treatment. For nondiseased-based parameters, control values are provided for reference using the letter C, although control values were excluded from analyses. The grey dashed line is a 1:1 reference line

(Figure 3b). For both seedling length and dry weight (data not presented), the greatest separation between methods occurred for Genesis 114, Rupali and Yorker and less separation among the more resistant genotypes, CICA1328, 04067-81-2-1-1(B) and ILWC246. Genotype-specific interactions also occurred among the chickpea × *C. echinospermum* crosses (CICA1328, 04067-81-2-1-1(B)) and the *C. echinospermum* line (ILWC246) for both seedling length and dry weight. For the time to express symptoms of chlorosis, the genotypes Rupali and Yorker could be divided into two groups with greater days to chlorosis for the peat and sand surface method only, but for Genesis 114 both this method and the sand surface method had greater days to chlorosis (Figure 3c). However, for the days to

TABLE 3 Genotype-by-isolate interaction glasshouse experiment. Isolate main effects for 11 chickpea genotypes (listed in Figure 2) in peat medium root-inoculated with eight *Phytophthora medicaginis* isolates for the parameters predicted days to chlorosis (average *SEM*), predicted days to canker (average *SEM*), Box-Cox (BC)-transformed stem canker length (mm), BC-transformed stem canker length relative to seedling length (Ca/stem), Gaussianized (GG)-transformed seedling length (mm) and BC-transformed seedling dry weight (g)

Isolate	Day Chlorosis (*SEM*)	Day Canker (*SEM*)	BC Canker length (bt)	BC Ca/stem (bt)	GG length (bt)	BC DW (bt)
5601	31.3 (2.85)	39.6 (3.70)	75.8 (19.2)	6.9 (11.4)	159.2 (161)	−1.26 (0.34)
7616	23.8 (2.03)	33.5 (2.88)	106.0 (23.5)	9.2 (15.7)	143.3 (143)	−1.64 (0.26)
7830	26.8 (2.56)	34.7 (2.96)	91.1 (21.6)	8.0 (13.2)	149.8 (153)	−1.26 (0.33)
7838	24.9[a] (2.20)	31.1[a] (2.52)	103.4 (22.5)	8.7 (15.3)	148.2 (147)	−1.50 (0.30)
7842	22.5 (1.84)	31.0 (2.43)	100.6 (23.1)	9.2 (15.4)	147.6 (147)	−1.61 (0.25)
7902	18.5 (1.51)	26.8 (2.05)	116.3 (25.4)	11.4 (19.7)	131.5 (129)	−2.35 (0.15)
7919	22.6 (2.00)	29.9 (2.54)	108.8 (23.7)	10.6 (18.6)	130.6 (127)	−1.94 (0.20)
9470	22.1 (1.81)	30.8 (2.41)	114.9 (25.2)	10.7 (18.5)	143.9 (143)	−1.92 (0.20)
SED/LSD			11.47/22.64	0.98/1.93	5.23/10.32	0.221/0.436

Note: Residual *df* = 174, *SED* and LSD values for analysis of variance and back-transformed (bt) values presented.

[a]No chlorosis or canker for genotype ILWC246.

TABLE 4 Phenotyping method experiment. Results for four phenotyping methods: peat medium surface inoculation (peat surface), peat medium root inoculation (peat root), peat and sand media surface inoculation (peat and sand surface) and sand medium surface inoculation (sand surface) for (a) parameters with significant ($p < 0.01$) phenotyping method main effects: predicted mean days to chlorosis and canker from survival analysis, water use over a 10 day period before the first dead plant occurred, canker length, log (Ln)-transformed seedling length and Box-Cox-transformed seedling dry weight (BC DW); and (b) significant ($p < 0.05$) phenotyping method-by-*Phytophthora medicaginis* isolate interactions among the two isolates (5601, 7842) for four parameters listed above

(a)

Method	Day to chlorosis (*SEM*)	Day to canker (*SEM*)	Water use (g/day)	Canker length (mm)	Ln seedling length (mm) (bt)	BC DW (g) (bt)
Peat surface	18.8 (1.15)	28.1 (1.61)	8.23	19.2	4.72 (134)	−2.03 (0.26)
Peat root	19.3 (1.18)	28.0 (1.54)	4.83	19.9	4.72 (135)	−1.94 (0.26)
Sand & peat surface	27.2 (1.67)	33.4 (1.74)	9.88	17.7	4.94 (154)	−1.12 (0.42)
Sand surface	22.9 (1.40)	32.1 (1.84)	5.29	11.2	4.49 (99)	−1.81 (0.27)
SED/LSD	–	–	0.099/0.197	1.73/3.42	0.029/0.057	0.114/0.225

(b)

	Day to chlorosis (*SEM*)		Canker length (mm)		BC DW g (bt)		Water use (g/day)	
Method/isolate	5601	7842	5601	7842	5601	7842	5601	7842
Peat surface	18.4 (1.12)	19.2 (1.17)	18.3	20.2	−2.04 (0.27)	−2.03 (0.26)	8.26	8.19
Peat root	17.8 (1.08)	20.9 (1.28)	20.5	19.2	−2.19 (0.24)	−1.68 (0.29)	4.52	5.15
Sand & peat surface	27.2 (1.67)	27.2 (1.67)	13.9	21.5	−0.97 (0.46)	−1.28 (0.39)	9.87	9.88
Sand surface	23.9 (1.46)	21.8 (1.33)	7.4	15.0	−1.64 (0.30)	−1.97 (0.24)	5.31	5.27
SED/LSD			2.45/4.84		0.161/0.318		0.141/0.278	

Note: For survival analysis values the average *SEM* for each value is included in parentheses, for analysis of variance output standard error of the differences of the means (*SED*) and least significant difference (LSD) values are presented, for transformed data back-transformed (bt) values are presented, residual *df* = 141.

canker interaction only Rupali had higher predicted days to canker values for the peat and sand surface method than the other methods, whereas for 04067-81-2-1-1(B) and ILWC246 values did not differ among methods (Figure 3d).

There was a significant phenotyping method-by-genotype-by-isolate interaction (M*G*I) for the parameter days to chlorosis (Table S2, Figure 3e).

3.4 | Isolate aggressiveness field experiment

The field experiment testing different isolate aggressiveness and G*I with six isolate treatments on two genotypes revealed that 10 of the 11 parameters measured were significant ($p < 0.05$) among isolate treatments (data not presented). Included in effects were differences among isolate mixtures and single isolates for significant isolate

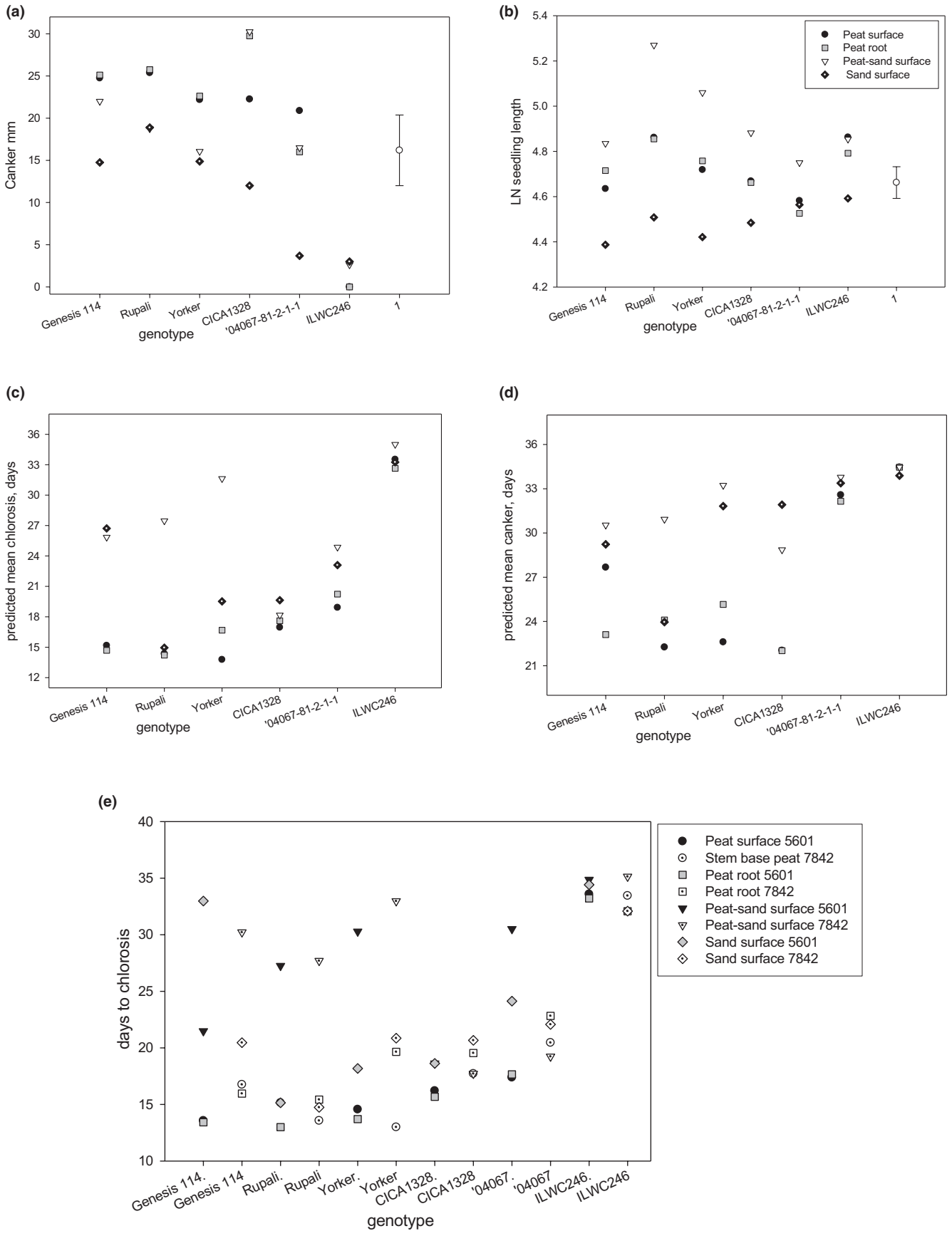
(a)
Canker mm
genotype
Genesis 114
Rupali
Yorker
CICA1328
'04067-81-2-1-1
ILWC246
1
(b)
LN seedling length
Peat surface
Peat root
Peat-sand surface
Sand surface
(c)
predicted mean chlorosis, days
(d)
predicted mean canker, days
(e)
days to chlorosis
Peat surface 5601
Stem base peat 7842
Peat root 5601
Peat root 7842
Peat-sand surface 5601
Peat-sand surface 7842
Sand surface 5601
Sand surface 7842

FIGURE 3 Phenotyping method experiment, method-by-chickpea genotype interactions for four methods (peat medium surface inoculation, peat surface; peat medium root inoculation, peat root; sand and peat media surface inoculation, peat-sand surface; sand medium surface inoculation, sand surface) and six genotypes for the parameters (a) canker length, (b) log (LN)-transformed seedling length, (c) predicted days to chlorosis (average *SEM* 1.35), (d) predicted days to canker (average *SEM* 1.74) and (e) method-by-genotype-by-*Phytophthora medicaginis* isolate (5601 or 7842) interaction for predicted mean days to chlorosis (average *SEM* 1.34, 04067 = 04067-81-2-1-1(B)). For analysis of variance output the least significant difference values for each interaction are presented as an error bar. Genotypes are ordered on the basis of established field resistance rankings, with the most susceptible genotypes to the left and most resistant to the right

treatment effects on emergence and seedling establishment. Isolates 5601, 7842 and the Equal Oospore isolate mixture (EqOosp) treatment all had lower emergence count values than the Equal Plate isolate mixture (EqPl) treatment. August seedling counts showed the same differences among treatments (Table 5). For the number of missing lesioned tap roots on plants, isolate 7842 had a higher value (7.1 plants) than all other isolates except 9470 (6.1 plants); isolate 9470 also had a higher value than isolates 4021 and 5601 (1.9 and 3.4 plants, respectively) but not either of the isolate mixture treatments (4.1 and 4.4 plants).

Eight of 11 parameters provided significant G*I; six examples are presented (Table 5). The AUDPS for dead plants only showed significant differences among isolate treatments for genotype Yorker, with isolate 7842 having a higher value than all other treatments. In addition, the EqOosp treatment had a higher AUDPS dead value than isolate 9470 (Table 5). The AUDPS dead values for treatments apart from 9470 were also higher in Yorker than those of 04067-81-2-1-1(B). In contrast, for the AUDPS Symp. parameter, all values were higher for Yorker than those of 04067-81-2-1-1(B). Again, for AUDPS Symp. parameters, values did not differ among 04067-81-2-1-1(B) isolate treatments. For Yorker isolate treatment values were in three groups: isolate 7842 had the highest value and the EqOosp treatment had the second highest value, which was higher than the remaining three single isolate treatments.

Further significant G*I effects were demonstrated for root and soil inoculum development parameters (Table 5). The total stem base–root DW interaction included single isolate and isolate mixture differences, for which values did not differ among 04067-81-2-1-1(B) isolate treatments, but on Yorker, isolate 7842 had the lowest value of all treatments

TABLE 5 Isolate aggressiveness field experiment. Results for the in-row inoculated at sowing chickpea genotypes Yorker and 04067-81-2-1-1(B) (04067) and six *Phytophthora medicaginis* isolate treatments including two isolate mixture treatments: equal oospore per isolate mixture (EqOosp.), equal no. plates isolate mixture (EqPl.) and a noninoculated control (not presented) for the parameters July emergence count; August seeding establishment count, square root (SQ)-transformed area under the disease progress stairs (AUDPS Dead) for dead plants, SQ-transformed area under the disease progress stairs (AUDPS Symp.) for plants with symptoms, total stem base–root weight (TSBR g/m^2), SQ-transformed number of *P. medicaginis* (Pm) DNA sequence copies/g of soil, kilo copies of soil Pm DNA relative to stem and root dry weight, and grain yields (kg/ha), residual *df* = 33. The percentage of total variance (PTV) for each interaction is provided after the LSD value

Parameter	Emerg. m^2	Seedling m^2	SQ AUDPS Dead		SQ AUDPS Symp.	
Genotype/isolate	Both genotypes	Both genotypes	04067	Yorker	04067	Yorker
4021	27.1	29.5	0.286	1.610	1.483	3.367
5601	25.7	27.8	0.511	1.253	1.856	3.387
7842	26.3	28.7	0.420	3.402	2.200	6.520
9470	28.0	30.5	0.548	1.073	1.830	3.606
EqPl.	28.8	31.2	0.199	1.192	1.603	3.623
EqOosp.	25.3	27.1	0.926	1.943	2.182	4.958
SED/LSD/PTV	1.56/2.25	1.45/2.09	0.3827/0.7786/24.4		0.4175/0.8495/10.6	

Parameter	TSBR weight[a]		SQ Pm DNA[a]		kPm DNA/g root[a]		Grain yield	
Genotype/isolate	04067	Yorker	04067	Yorker	04067	Yorker	04067	Yorker
4021	32.0	23.5	48	208	62	1695	2429	2626
5601	34.8	28.7	185	429	839	6976	2627	2167
7842	34.2	9.3	136	280	451	7691	2844	896
9470	34.8	20.5	200	712	1136	20,455	2867	2845
EqPl.	30.7	17.3	155	463	704	10,474	2586	2510
EqOosp.	32.4	15.9	119	323	404	6351	2706	1638
SED/LSD/PTV	3.58/7.29/10.3		60.3/122.7/10.5		2010.4/4090.2/18.7		304.4/619.2/29.7	

[a]Results for row sections sampled 13 November.

except for the EqOosp treatment, the EqOosp treatment then had lower values than both the isolate 4021 and 5601 treatments. The extent of soil inoculum development was one of the only parameters that differed among isolate treatments on 04067-81-2-1-1(B), whereby the least aggressive isolate 4021 had less inoculum than both isolates 5601 and 9470, but these differences in inoculum development on 04067-81-2-1-1(B) were not evident for soil DNA concentrations relative to root dry weight. For differences in soil inoculum development on Yorker, isolate 9470 had higher values than all other isolates for both soil DNA concentration and DNA concentrations relative to root dry weight, but the rankings of other isolates differed among the two soil DNA parameters. For example, isolate 7842 had lower soil inoculum concentrations than the EqPl treatment and isolate 5601, but due to low 7842 root weights, the DNA concentrations relative to root dry weight of isolate 7842 did not differ from those of the EqPl treatment and isolate 5601. Differences in aggressiveness effects of single *P. medicaginis* isolates on the yield of Yorker were evident, with the yield of the isolate 7842 treatment lower than all other single isolates.

In addition to the differing effects of isolate mixture treatments on emergence and seedling numbers, disease symptoms expressed as AUDPS Symp. values were higher for the EqOosp treatment than the EqPl treatment on Yorker (Table 5). Differences among the isolate mixture treatments continued for both soil DNA concentration and DNA concentrations relative to root dry weight, whereby the EqPl treatment had higher inoculum values than the EqOosp treatment. Grain yields also differed between these mixture treatments where the EqPl treatment with higher inoculum values had a higher yield than the EqOosp treatment with lower inoculum values.

3.5 | Correlation between glasshouse and field experiment

Comparisons were carried out to test if G*I performance on genotypes and isolates common to genotype-by-isolate glasshouse and isolate aggressiveness field experiments differed. Comparison of results for three isolates (5601, 7842 and 9470) on the two genotypes (04067-81-2-1-1, Yorker) common to both experiments provided seven significant correlations (Table S3). The seven correlations were based on either the glasshouse experiment number of days to chlorosis or from chlorosis to canker, against the field AUDPS dead or plant symptom values, average stem base–root weight, total stem base–root weight, *P. medicaginis* DNA/g soil and grain yields. Examples of correlations based on either the glasshouse experiment number of days to chlorosis or from chlorosis to canker and field parameters are presented in Figure 4.

4 | DISCUSSION

Results demonstrated a significant range in *P. medicaginis* isolate aggressiveness reactions and parameter-specific G*I across 37 individual isolates on two media surface-inoculated chickpea genotypes, including a chickpea × *C. echinospermum* cross. A second glasshouse experiment also showed differing isolate aggressiveness and parameter-specific G*I across 11 genotypes including two chickpea × *C. echinospermum* crosses. A comparison of phenotyping methods showed M*G including reactions with chickpea × *C. echinospermum* crosses and M*I across multiple parameters. A field experiment demonstrated significant differences in *P. medicaginis* isolate aggressiveness among a small set of isolates.

Our test for differing *P. medicaginis* isolate aggressiveness reactions across 37 individual isolates on two genotypes provided clear evidence for a continuum in aggressiveness. The findings for differing *P. medicaginis* isolate aggressiveness on chickpea and lucerne by previous studies (Du et al., 2013; Liew & Irwin, 1997) was supported by our results for the chickpea cv. Rupali. Our results for differing *P. medicaginis* isolate aggressiveness reactions on a chickpea × *C. echinospermum* cross will be of relevance to chickpea breeding practices, as chickpea × *C. echinospermum* crosses are the key source of *P. medicaginis* resistance (Amalraj et al., 2019; Knights et al., 2008). Both *P. medicaginis* isolate canker length and canker length relative to stem length parameters differed among 37 isolates, confirming previous studies for differences in recognized quantitative traits such as lesion size as important components of isolate aggressiveness (Pariaud et al., 2009). Variation in oomycete isolate reactions may be due to a number of factors including the concentration of inoculated propagules (Palloix et al., 1988), selection pressure effects under low diversity crop rotations (Zhang & Yang, 2000), the nutritional status of an isolate affecting development (Nelson & Hsu, 1994), and isolates having different optimal temperatures for disease development (Rossman et al., 2017). Our oospore inoculation method provided control over oospore concentrations but not contributions from mycelial fragments. We did not investigate what factors are responsible for differing isolate reactions but there were differences among *P. medicaginis* isolates in traits such as oospore production.

Mixtures of isolates can be used in breeding programmes to select for a broad range of resistances against a variable pathogen population. For example, isolates of *Phytophthora sojae* that differed in virulence on *Rps* genes were combined in a mixture to screen soybean lines for novel *Rps* genes (Matthiesen et al., 2016). A mixture of 10 *P. medicaginis* isolates combined on an equal oospore concentration basis in a glasshouse and field experiment was not more aggressive than that of individual aggressive isolates. In addition, our field-based mixture comparison indicated a larger contribution of oospores from weakly aggressive isolates for an equal plate per isolate mixture provided a depression in isolate mixture aggressiveness, relative to an equal oospore number per isolate mixture. A mixture of 10 *P. medicaginis* isolates has been used for resistance screening in the Australian Chickpea Breeding Program (Amalraj et al., 2019). The use of mixtures with disproportional contributions of weakly aggressive isolates may lead to moderated disease reactions; however, the use of isolate mixtures avoids the risk of selecting for isolate-specific responses.

Three of our experiments provided evidence for G*I. The peat medium surface inoculation-based aggressiveness range and in-row inoculated at sowing field experiments demonstrated a propensity for G*I in this *P. medicaginis*–chickpea pathosystem

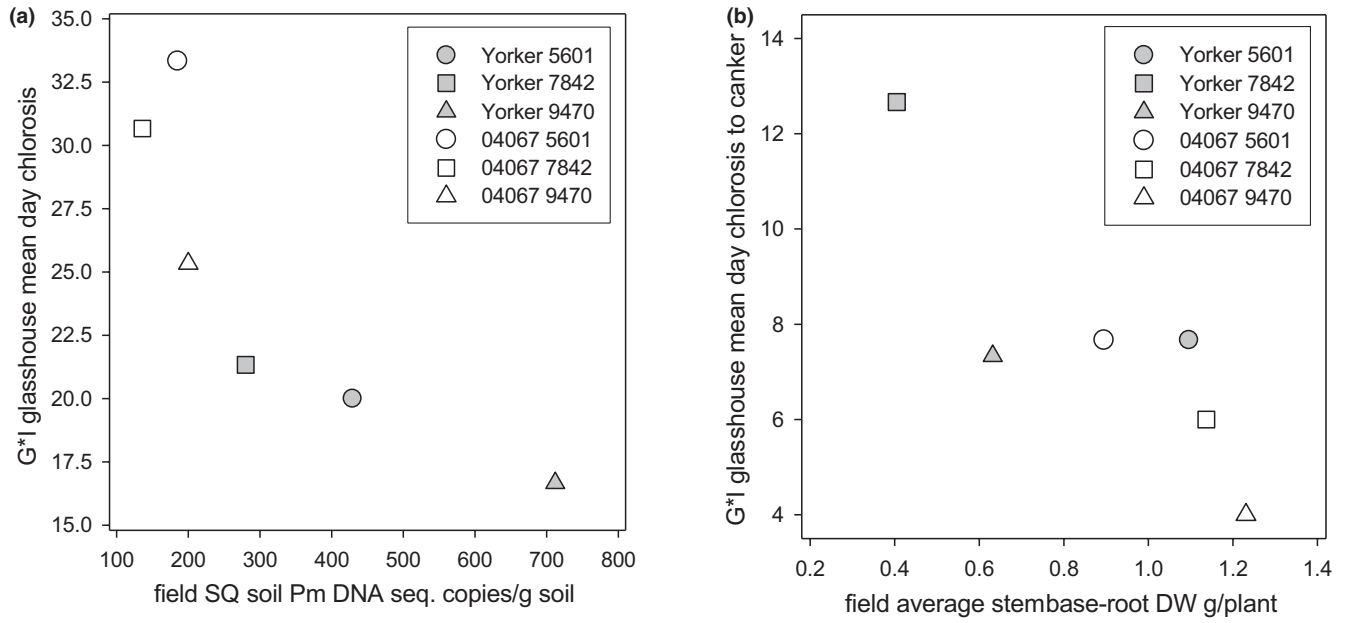

FIGURE 4 Plots of the in-row at sowing inoculated isolate aggressiveness field (Field) and root-inoculated genotype-by-isolate interaction glasshouse (G*I) experiments, results for the chickpea genotypes Yorker and 04067-81-2-1-1 (04067) with common *Phytophthora medicaginis* isolate (5601, 7842 and 9470) treatments with significant ($p < 0.05$) correlations among (a) field soil square root-transformed (SQ) *P. medicaginis* inoculum concentrations and glasshouse days to chlorosis (r −0.84) and (b) field average stem base–root dry weight (DW) and glasshouse days from chlorosis to canker (r −0.86)

among only two genotypes of contrasting resistance. The field experiment results demonstrated multilevel effects including foliage symptoms, root development, inoculum production and grain production parameter interactions. The aggressiveness range and G*I glasshouse experiments and the field experiment all provided parameter-specific G*I. The field experiment included some differences in isolate aggressiveness reactions on a *C. echinospermum* backcross; however, the two glasshouse experiments provided G*I with a wider range of isolate aggressiveness reactions on the *C. echinospermum* crosses than the field experiment. A wide range of isolate aggressiveness reactions was also observed on a pure *C. echinospermum* line for the parameter canker length relative to stem in the G*I interaction glasshouse experiment, whereas a narrow range in isolate reactions occurred on three susceptible chickpea genotypes. These findings are supported by Faris (1985) who reported G*I in the *Phytophthora megasperma*–lucerne pathosystem, and found the most similarity in disease reactions among isolates on susceptible lucerne genotypes. In our study the percentage of variance accounted for by G*I ranged from 10% to 30% across all experiments, thus when interactions do occur in our pathosystem they are not minor in scale. Studies that followed Van der Plank's (1963) observations on the implications between G*I in quantitative systems have concluded that the detection of a significant G*I may not necessarily indicate host–isolate specificity (Carson, 1987; Pariaud et al., 2009). As the phenotyping environment is an important factor affecting interactions between isolates and genotypes (Kulkarni & Chopra, 1982), we needed to evaluate in a single experiment the variance among phenotyping methods and effect on G*I.

Erwin (1966), Pratt and Mitchell (1976) and Irwin and Dale (1982) reported single phenotyping methods on *P. megasperma* that showed differences among isolates and genotypes but no G*I, whereas Faris (1985), Liew and Irwin (1997) and Du et al. (2013) provided evidence for *P. megasperma* or *P. medicaginis* host G*I from studies using a single phenotyping method. Our comparison of four phenotyping methods across two isolates provided no significant G*I interactions; rather, results provided multiple parameter-specific M*G and M*I interactions. Two significant main effects for method also accounted for more than 40% of the total variance. Although only two isolates were compared in this experiment, they provided M*I for four of eight parameters, and for the six chickpea genotypes there were six M*G among eight parameters. Although M*I occurred it was also notable that for a number of parameters the most aggressive isolate (7842) was less affected by methods than the less aggressive isolate (5601); similar findings have been reported in other pathosystems (Jin & Shew, 2021; Matthiesen & Robertson, 2021). For the parameter days to chlorosis there was a significant M*G*I, whereby there were differing *P. medicaginis* isolate reactions among phenotyping methods for two contrasting genotypes. Our results provide evidence that phenotyping methods affect both host genotype reactions and associated resistance rankings and isolate performance, including the effect of phenotyping method on G*I.

We identified a number of *P. medicaginis* isolates and phenotyping methods that are useful for resistance breeding. Findings for isolate reactions on a *C. echinospermum* line in the root-inoculated G*I interaction glasshouse experiment indicated that the most aggressive *P. medicaginis* isolates could be selected from those that cause the most disease on the most resistant hosts.

There was support for this principle from studies of the pathogenicity of *Phytophthora infestans* isolates on potato varieties with race-nonspecific resistance (Young et al., 2018). Dale and Irwin (1991b) showed in chickpea genotypes with differing resistance to *P. megasperma* that the growth rate of *P. megasperma* was slower in root tissue of the partially resistant genotype but did not differ among genotypes in the epicotyl. They concluded that chickpea phenotyping systems that promote infection of the epicotyl will not provide identification of genotypes that express resistance in the roots. The development of root infection-based phenotyping systems has been shown to be important for identifying partial resistance to a range of *P. sojae* isolates in soybean in contrast to hypocotyl-based systems for race-based resistance phenotyping (Karhoff et al., 2019). In our experiments, correlations between the root-inoculated G*I interaction glasshouse and in-row-inoculated at sowing field experiment results provided a small number of significant relationships based on related G*I. The negative relationship between glasshouse days to chlorosis and soil inoculum development indicated that genotype–isolate combinations with slow glasshouse chlorosis symptom development related to low field inoculum development and vice versa. However, concentrations of *P. medicaginis* inoculum in soil are not stable, with inoculum decline occurring after peak disease events in susceptible chickpea varieties (Bithell et al., 2021), thus genotype–isolate soil inoculum concentration is expected to be time point-dependent. The comparison of the peat medium-based root and surface inoculation in the phenotyping method experiment showed that root inoculation provided more rapid and severe disease symptoms for isolate 5601. Together these findings indicate that root inoculation-based methods may provide advantages in terms of challenging chickpea genotypes with a method that provides early foliage symptoms, ability to cause severe reductions in dry weight and can produce G*I reactions that relate to field G*I reactions.

In conclusion, we found evidence for differences in the aggressiveness of *P. medicaginis* isolates across a large set of isolates; G*I occurred among eight isolates on 11 genotypes; G*I also occurred in field-based reactions among six isolate treatments on two genotypes. The composition of isolate mixtures affected genotype resistance reactions. Different phenotyping methods provided both M*G and M*I across multiple parameters, and the ability for M*G*I, although only for one parameter. All experiments provided differing isolate aggressiveness reactions on chickpea × *C. echinospermum* crosses. Overall, our results showed that to achieve genetic gain for *P. medicaginis* resistance improved phenotyping methods are required; the current studies are an important step towards this end.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from New South Wales Department of Primary Industries. Restrictions apply to the availability of these data, which were used under co-ownership with the Grains Research and Development Corporation, Australia for this study. Data are available from the authors at New South Wales Department of Primary Industries with the permission of Grains Research and Development Corporation, Australia.

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SUPPORTING INFORMATION

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